QUATERNARY ALKALOIDS FROM THE ROOTS OF *Argemone platyceras* LINK *et* OTTO*

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From the fraction of quaternary alkaloids obtained from the roots of *Argemone platyceras* LINK *et* OTTO (−)-α-stylopine methohydroxide, (−)-α-canadine methohydroxide, magnoflorine, cyclanoline and platycerine methohydroxide were isolated in the form of iodides or perchlorates and the presence of trace amounts of an alkaloid, probably identical with argemonine methohydroxide, and a small amount of four additional unidentified alkaloids was demonstrated. A convenient method of separation of non-phenolic and phenolic quaternary alkaloids is also described.

We described quaternary alkaloids from the overground parts of *Argemone platyceras* LINK *et* OTTO recently[1]. After conversion to perchlorates we isolated platycerine methoperchlorate as the main component of the quaternary fraction and smaller amounts of (−)-argemonine methoperchlorate and (−)-stylopine methoperchlorate which consisted predominantly of the α-form in addition to a smaller amount of the β-form[2]. In the present investigation we focussed our attention on quaternary alkaloids from the root of the same plant species. In contrast to the overground part which contains only a negligible part of quaternary bases (0·003%), the root contains an unusually high concentration of this alkaloid fraction (0·14% of the dry material). After separation of the "non-quaternary" fraction of bases which can be extracted at alkaline reaction with ether (fractions *A* and *B*) or chloroform (fraction *E*), quaternary alkaloids were obtained in the form of iodides in the usual manner, as in paper[1]. For the isolation of individual alkaloids from the crude mixture of iodides the separation of this fraction to iodides of non-phenolic alkaloids (I_1) and phenolic ones (I_2) using a procedure described in the experimental part was found suitable.

The main constituent of the non-phenolic fraction of iodides (I_1) was (−)-α-stylopine methiodide the properties of which were described recently[2]. The root of *A. platyceras* represents so far the richest source of this relatively rare alkaloid. In contrast to the overground part the presence of the diastereoisomeric β-form could not be detected in the root. (−)-α-Canadine methiodide which could not be detected

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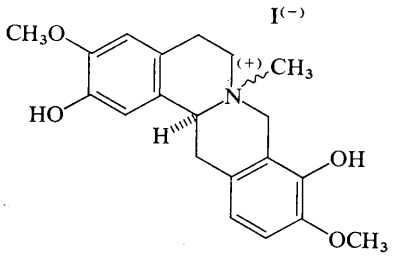
in the overground part of the plant was isolated in a lower yield from the root and the presence of trace amounts of an alkaloid, probably identical with argemonine methiodide, was also demonstrated in it by thin-layer chromatography.



I

From the phenolic fraction I_2 the iodide of an alkaloid was isolated which was identified on the basis of its melting point, mixed melting point, mass spectrum, ^{1}H-NMR, IR and UV spectra, and R_F values as cyclanoline iodide —(−)-α-scoulerine methiodide[3] (*I*). This is the first case when the alkaloid was found in *Papaveraceae*. Diastereoisomeric (−)-β-scoulerine methohydroxide was found recently in negligible amount in *A. albiflora* HORNEM.[4] and *A. mexicana* L.[5] Cyclanoline represents the main alkaloid of the quaternary fraction of the roots of *A. platyceras*. As an additional alkaloid from the phenolic fraction magnoflorine iodide was also isolated. This alkaloid, very common in many plant families, has not yet been found in the *Argemone* genus. From the amorphous residue of the phenolic quaternary alkaloids after the conversion to perchlorates platycerine methoperchlorate was isolated by column chromatography and the presence of a small amount of four additional unidentified alkaloids was demonstrated.

It was observed that some of the mentioned quaternary alkaloids (α-stylopine methohydroxide, α-canadine methohydroxide, platycerine methohydroxide) pass partly into chloroform even with anions different from iodide (probably with chloride anion) when isolated in the described manner; thus they come into fraction *E* from where they may be obtained and separated from the "non-quaternary" base present in this fraction using the procedure described in the experimental part.

EXPERIMENTAL

The melting points were determined on a Kofler block and they are not corrected. The mass spectra were measured on an AEI-MS 902 mass spectrometer, the ^{1}H-NMR spectra on a Varian T-60 instrument, using tetramethylsilane as internal standard, the IR spectra were recorded with an Infrascan (Hilger and Watts) spectrophotometer, and the UV spectra (in methanol) with a Unicam SP 1800 apparatus. For thin-layer chromatography silica gel G Merck with gypsum

(5 : 1) was used in combination with the following systems: ethanol–water–25% ammonia 15 : 9 : 1 (S_1), methanol–water–25% ammonia 15 : 3 : 1 (S_2) and 5 : 1 : 1 (S_3), and 1-propanol–water–formic acid 12 : 7 : 1 (S_4). Detection was carried out with potassium iodoplatinate. Paper chromatography was carried out on Whatman No. 1 paper, descending manner, with 1-butanol–acetic acid–water 10 : 1 : 3 (S_5), using Dragendorff's reagent for detection.

Extraction and Isolation of Alkaloids

The plants were cultivated in the Experimental Botanical Garden of the Medical Faculty in Brno from the seeds obtained from botanical gardens in Bonn, Gattersleben and Karlsruhe. They were gathered on September 5th, 1967 and September 18th, 1969. The roots were separated from the overground parts and dried at room temperature. Dry, ground roots (4·31 kg) were extracted with ethanol in a Soxhlet extractor. After evaporation of ethanol the extract was dissolved in 1% sulfuric acid and extracted with one litre of ether in order to eliminate lipoid substances. From the aqueous layer the alkaloidal fraction *A* (30·87 g of bases: 0·71%), *B* (0·11 g of chlorides: 0·003%; predominantly coptisine, a smaller amount of berberine and corysamine), *E* and *I* were obtained in the same manner as in paper[1].

Fraction I (quaternary alkaloid iodides) was dissolved in boiling water and the solution was filtered with active charcoal, cooled and alkalized with sodium hydroxide solution. A solution of 20 g of potassium iodide was added and the mixture was extracted four times with chloroform into which the iodides of non-phenolic quaternary alkaloids (I_1) had passed. The aqueous layer was acidified weakly with hydrochloric acid and extracted several times with chloroform or chloroform containing 20% ethanol. Iodides of phenolic quaternary alkaloids (I_2) were thus obtained. From fraction I_1 0·76 g of the poorly soluble (−)-α-stylopine methiodide (total yield 1·24 g; 0·029%) and 0·10 g of the better soluble (−)-α-canadine methiodide (total yield 0·34 g; 0·008%) were obtained by crystallizations from methanol. The mother liquors contained 0·05 g of amorphous iodides among which in addition to the remainders of the two mentioned alkaloids, a small amount of magnoflorine, platycerine methosalt and cyclanoline, the presence of another alkaloid was proved, which — according to its R_F values (0·25 in S_1 and 0·06 in S_2) — is probably identical with argemonine methiodide.

Fraction I_2 gave magnoflorine iodide (0·64 g; 0·015%) on crystallization from methanol. From the strongly concentrated methanolic mother liquor cyclanoline iodide crystallized out after several weeks' standing. After recrystallization from water the yield was 2·04 g (total content in the root is about 2·24 g; 0·052%). The non-crystallizing residue of iodides was purified by conversion to poorly soluble perchlorates (1·20 g) which, however, also remained amorphous. A part of these perchlorates (0·40 g) was separated on a column of alumina (60 g), which was prewashed with 1% perchloric acid and dried at 210°C (see[1]). The column was prepared in chloroform, the amorphous perchlorates were dissolved in 1·6 ml of methanol, 16 ml of chloroform were added, and the mixture was chromatographed. For elution chloroform was used first (100 ml), followed by chloroform containing an increasing amount of methanol: 2·5% (250 ml), 5% (100 ml), 10% (300 ml), 15% (100 ml), 20% (300 ml), 30% (300 ml), 50% (500 ml). The column was finally washed with methanol (100 ml). Fifty ml fractions were collected (with the exception of fractions 1 and 39, which were of 100 ml volume) and their composition was controlled by thin layer chromatography in S_1 and S_2. Fractions 1 to 7 (total 7·5 mg) were non-alkaloidal, fractions 8 to 12 (74·6 mg) contained pure platycerine methoperchlorate (total yield in the root about 0·010%), fractions 13 to 14 (8·8 mg) contained in addition to platycerine methosalt a small amount of an alkaloid with R_F 0·36 or 0·15, respectively. Fractions 15 to 17 (34·5 mg) contained in addition to both these alkaloids another alkaloid of R_F 0·52 or 0·36, respectively, and traces of

an alkaloid with R_F 0·15 (S_1). Fractions 18 to 20 (48·2 mg) contained an alkaloid of R_F value 0·59 or 0·41 respectively and cyclanoline, fractions 24 to 39 consisted of practically pure cyclanoline (after conversion to iodide their weight was 40·3 mg).

The crude fraction E was dissolved in 5% sulfuric acid, the solution was alkalized with ammonia and extracted with 3 portions of chloroform (fraction *EE*, 3,23 g). The aqueous layer was acidified weakly with hydrochloric acid, a solution of 20 g of potassium iodide was added and the mixture was extracted again with several portions of chloroform (fraction *EI*). Crystallizations of fraction *EI* from methanol led to the separation of (—)-α-stylopine methiodide (0·45 g) and (—)-α-canadine methiodide (0·19 g). The noncrystallizing residue of the iodides was separated to iodides of non-phenolic (EI_1) and phenolic (EI_2) alkaloids in the above mentioned manner. Crystallization of fraction EI_1 (0·13 g) from methanol also gave 0·03 g of (—)-α-stylopine methiodide and 0·05 g of (—)-α-canadine methiodide. In the amorphous residue (0·06 g) a small amount of an alkaloid, probably identical with argemonine methiodide was detected by thin layer chromatography in S_1 and S_2 in addition to the two alkaloids mentioned. From the amorphous fraction EI_2 (0·32 g) perchlorates (0·23 g) were prepared by precipitation of the aqueous solution with 20% sodium perchlorate solution, but also remained amorphous. According to thin-layer chromatography (S_1 and S_2) they consisted almost exclusively of platycerine methoperchlorate with traces of an alkaloid which had R_F 0·74 in S_1 and 0·42 in S_2. The preparation obtained by precipitation with ether of the methanolic solution had m.p. 155—162°C.

Characterization of Alkaloids

(—)-α-*Stylopine methiodide*: m.p. 279—281°C (methanol), undepressed on admixture of an authentic sample[2], $[\alpha]_D^{23}$ −127° ±3° (*c* 0·17, methanol). The UV spectrum (see[2]), R_F values (0·45 in S_1, 0·20 in S_2 and 0·71 in S_5) and characteristic colour reactions were identical with those of an authentic sample (R_F values of the β-form: 0·50 in S_1 and 0·58 in S_5).

(—)-α-*Canadine methiodide*: leaflets from methanol, m.p. 160—162°C, at about 180°C the melt solidified to a crystalline mass and remelted at 210—215°C, at 225°C it again solidified to needles (transformation to β-form), which again remelted at 249—255°C (decomposition). The same behaviour was also displayed by the mixture with an authentic sample (see[6]). $[\alpha]_D^{23}$ −115° ±2° (0·36, methanol). The UV spectrum[6] and the R_F values, 0·55 (S_1) and 0·75 (S_5), were also identical with the data for an authentic sample.

Magnoflorine iodide: m.p. 265—266°C (methanol), mixed melting point with authentic sample[7] was undepressed, $[\alpha]_D^{24}$ 187° ±5° (*c* 0·16, methanol). The UV spectrum and R_F values, 0·65 (S_1), 0·42 (S_2), 0·52 (S_3), 0·53 (S_4) and 0·46 (S_5), were identical with those of an authentic sample.

Cyclanoline iodide: needles, m.p. 167—169°C (water or methanol), undepressed in admixture with an authentic preparation,* easy soluble in methanol, poorly soluble in cold water, well soluble in boiling water, $[\alpha]_D^{22}$ −100° ±3° (*c* 0·37, methanol). Literature[3] gives the value for chloride only: $[\alpha]_D^{22}$ −116° (methanol). Mass spectrum: molecular ion of the tertiary base formed on pyrolysis had mass 327·1470 (for $C_{19}H_{21}NO_4$ calculated 327·1470); characteristic peaks at *m/e* 178 (base peak), 176, 150, 149, 142 (CH_3I), 135, 127 (I) (see[4]). ^{1}H-NMR spectrum (in dimethyl sulfoxide): δ 3·27 p.p.m. (N—CH_3), 3·85p.p.m. (2 OCH_3), the signal at 4·74 p.p.m. (N—CH_2—Ar) is overlapped by the multiplet (angular H), four aromatic protons (two isolated, two *ortho*), doublet at 6·75 p.p.m., J_{ortho} = 8·5 Hz (1 H), singlet at 6·77 p.p.m. (1 H), singlet at 6·92 p.p.m.

* For comparison a sample of cyclanoline iodide was used which was kindly supplied by Prof. Dr M. Tomita, Kyoto, Japan.

(1 H), and doublet at 7·05 p.p.m., $J_{ortho} = 8{\cdot}5$ Hz (1 H). The IR spectrum (in nujol) and the UV spectrum, λ_{max} (log ε) 285 mm (3·94), λ_{min} 257 nm (3·47), were identical with the spectra of the authentic sample. The same is true of R_F values: 0·72 (S_1), 0·48 (S_2), 0·56 (S_3), 0·71 (S_4) and 0·61 (S_5).

Platycerine methoperchlorate: the amorphous product partly crystallized after gradual evaporation of a methanolic solution; prisms in clusters, m.p. sharply at 165°C, very easy soluble in methanol. A preparation obtained by precipitation of a methanolic solution with ether (see[1]) had m.p. 160—164°C, undepressed on admixture of a sample prepared from platycerine[1]. $[\alpha]_D^{23}$ $-258° \pm 3°$ (*c* 0·47, methanol) in agreement with the literature data[1]. The UV spectrum (see[1]) and the R_F values (0·65 in S_1, 0·31 in S_2, 0·50 in S_3, 0·61 in S_4 and 0·71 in S_5) were identical with those of an authentic preparation.

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